Litton BIONETICS

Final report-Mutagenicity Evaluation of Powdered Guaiac Resin FDA 75-66 4/77

MUTAGENICITY EVALUATION

OF

POWDERED GUAIAC RESIN
FDA 75-66

FINAL REPORT

5516 Nicholson Lane
Kensington, Maryland
20795

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FDA 75-66

FINAL REPORT

SUBMITTED TO

FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
5600 FISHERS LANE
ROCKVILLE, MARYLAND 20852

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

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Litton BIONETICS

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EVALUATION SUMMARY

The test compound, Powdered Guaiac Resin, FDA 75-66, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: April, 1977

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound Powdered Guaiac Resin, FDA 75-66

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: October 29, 1976

2. Description: Black powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae*, strain D4

Bacteria Strains: *Salmonella typhimurium*, strains TA-1535, TA-1537, TA-1538, TA-98, TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	4 µmoles
2. Glucose-6-phosphate	5 µmoles
3. Sodium phosphate (dibasic)	100 µmoles
4. $MgCl_2$	8 µmoles
5. KCl	33 µmoles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS[b]
	Ethylmethanesulfonate	Water or saline	BPS[b]
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard	Water or saline	FS[b]
Activation	Dimethylnitrosamine	Water or saline	BPS[b]
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline	Dimethylsulfoxide[c]	FS[b]
	2-Aminoanthracene	Dimethylsulfoxide[c]	BPS[b]

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: Powdered Guaiac Resin FDA 75-66

2. Test solvent: DMSO

3. Solubility of the test compound under treatment conditions:
Soluble under test conditons.

4. Additional comments: Black powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination:

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

	Percent Concentration	
Test Doses	Bacteria	Yeast
1/4 50% Survival	0.055	0.3125
1/2 50% Survival	0.11	0.625
50% Survival	0.22	1.25

C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.

SUMMARY OF TEST RESULTS

PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: PM9000297

B. TEST DATE: NOV. 30, 1976

TEST		SPECIES	TISSUE	REVERTANTS PER PLATE									
				TA-1535		TA-1537		TA-1538		TA-98		TA-100	
				1	2	1	2	1	2	1	2	1	2
1. NON-ACTIVATION													
SOLVENT CONTROL*		---	---	19	26	12	22	28	17	58	43	145	110
POSITIVE CONTROL**		---	---	>1000	>1000	>1000	460	>1000	>1000	>1000	>1000	>1000	>1000
TEST	0.22000 %	---	---	10	11	10	11	17	14	39	32	101	91
	0.11000 %	---	---	21	24	11	12	20	23	66	53	120	88
	0.05500 %	---	---	19	15	22	13	27	15	40	49	114	138
2. ACTIVATION													
SOLVENT CONTROL*		MOUSE	LIVER	17	18	30	33	27	25	57	67	154	128
		RAT	LIVER	35	16	22	20	20	13	72	58	215	206
		MONKEY	LIVER	32	32	23	35	41	36	80	107	221	267
POSITIVE CONTROL***		MOUSE	LIVER	111	110	210	187	>1000	>1000	>1000	>1000	>1000	>1000
		RAT	LIVER	59	65	685	675	289	276	617	631	688	658
		MONKEY	LIVER	107	198	634	467	430	302	623	452	>1000	>1000
TEST	0.22000 %	MOUSE	LIVER	10	10	16	12	14	15	50	49	145	159
	0.11000 %	MOUSE	LIVER	11	13	26	23	16	16	56	62	160	213
	0.05500 %	MOUSE	LIVER	26	11	27	26	15	11	35	42	192	144
	0.22000 %	RAT	LIVER	18	16	16	18	11	4	44	41	169	146
	0.11000 %	RAT	LIVER	17	24	27	22	16	18	64	43	187	151
	0.05500 %	RAT	LIVER	35	19	24	27	13	10	57	64	194	132
	0.22000 %	MONKEY	LIVER	11	12	19	34	14	16	27	40	186	167
	0.11000 %	MONKEY	LIVER	29	15	33	40	22	15	64	55	174	180
	0.05500 %	MONKEY	LIVER	46	41	19	37	18	33	68	54	240	165

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535 MNNG 2 UG/PLATE
TA-1537 QM 20 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 2 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/12/77

NONACTIVATION COMPOUND PM9000297

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
NAN		56.09	5.34	20.06	10.96	5.39	6.84	8.58	CONTROLS
NAP		164.41	587.36	137.92	187.12	143.51	249.25	212.69	
NA1		43.96	5.10	20.29	9.93	7.11	12.60	7.36	TEST DATA
NA2		48.42	5.91	14.30	9.86	4.22	8.14	10.98	
NA3		47.49	3.40	14.90	10.00	3.33	8.57	5.71	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/12/77

SPECIES ICRFLO/MOUSE COMPOUND PM9000297

TEST	ORG	TA100 HIS EX-A	TA100 HIS EX-A	TA1535 HIS EX-A	TA1537 HIS EX-A	TA1538 HIS EX-A	TA98 HIS EX-A	000004 ADE EX-5	000004 TRY EX-5	
ACT	A+C	43.20	59.24	9.01	4.23	12.17	2.55	9.55	4.36	NEGATIVE CONTROLS
ACT	A-C	32.56	47.18	8.33	2.38	7.39	2.87	9.50	1.67	
ACT	ALI	31.93	45.01	11.11	3.28	10.83	5.80	20.64	8.85	
ACT	ALU	44.37	74.78	8.72	5.65	11.66	4.99	6.36	1.54	
ACT	PLI	37.00	105.10	179.78	67.15	133.72	58.00	60.83	54.32	POSITIVE CONTROLS
ACT	PLU	27.32	57.36	11.78	1.83	42.72	24.95	8.81	4.02	
ACT	LII	******	62.72	8.03	6.14	6.21	18.69	8.99	6.16	TEST COMPOUND
ACT	LI2	66.20	72.01	5.62	1.81	6.58	5.02	14.40	8.13	
ACT	LI3	39.66	47.68	4.90	1.95	10.30	4.58	5.53	3.04	
ACT	LU1	******	54.66	3.31	4.13	10.53	10.03	13.74	4.87	
ACT	LU2	19.34	58.42	4.50	1.82	7.80	6.78	8.93	4.94	
ACT	LU3	36.88	63.59	4.32	1.99	13.49	3.85	9.44	5.84	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/12/77

SPECIES SPRDAW/RAT COMPOUND PM9000297

TEST	ORG	TA100 HIS EX-A	TA100 HIS EX-A	TA100 HIS EX-A	TA1535 HIS EX-A	TA1537 HIS EX-A	TA1538 HIS EX-A	TA98 HIS EX-A	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	45.96		134.75	6.79	10.81	23.31	3.75	6.88	5.37	NEGATIVE CONTROLS
ACT	A-C	49.21		104.45	8.71	3.14	10.64	3.67	6.33	4.13	
ACT	ALI	49.80	71.54	138.36	6.91	3.60	6.37	10.48	12.69	5.55	
ACT	ALU	52.54	64.72	131.52	6.93	6.99	6.71	6.43	8.77	4.94	
ACT	PLI	46.44	75.65	160.34	85.50	44.74	23.28	109.88	74.65	64.30	POSITIVE CONTROLS
ACT	PLU	38.92		96.69	6.38	5.64	566.01	86.28	8.88	3.84	
ACT	LI1	******	52.93	71.11	4.57	8.24	8.78	17.52	11.24	4.24	TEST COMPOUND
ACT	LI2	51.95		82.20	5.38	1.47	4.03	6.71	5.11	3.97	
ACT	LI3	47.52		104.65	2.99	1.23	8.66	6.99	6.46	3.71	
ACT	LU1	******	64.65	126.57	3.68	2.65	10.72	6.53	11.10	4.20	
ACT	LU2	140.48	50.71	85.28	7.33	2.57	3.34	3.69	7.25	4.99	
ACT	LU3	47.58		104.32	5.74	2.12	3.73	4.75	8.10	6.48	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/12/77

SPECIES RHESUS/MONKEY COMPOUND PM9000297

TEST	ORG	TA100 HIS EX-8	TA100 HIS EX-8	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	51.32		123.00	9.02	2.18	9.33	3.68	6.24	4.08	NEGATIVE CONTROLS
ACT	A-C	32.77		85.82	2.67	8.66	7.57	5.62	4.91	4.70	
ACT	ALI	45.20	31.66	91.19	2.17	3.79	7.46	13.68	7.60	8.01	
ACT	ALU	45.52	28.71	104.96	3.46	4.18	8.33	7.27	6.58	5.25	
ACT	PLI	39.48	82.52	218.23	117.77	72.02	224.19	100.30	51.53	50.43	POSITIVE CONTROLS
ACT	PLU	41.41		79.80	45.13	3.61	10.40	8.69	6.23	4.20	
ACT	LI1	******	67.89	98.62	3.21	5.01	10.81	12.65	9.94	6.73	TEST COMPOUND
ACT	LI2	73.16		89.03	2.55	2.54	10.12	4.15	5.58	5.85	
ACT	LI3	38.32		81.68	1.78	2.24	10.49	6.27	3.78	0.22	
ACT	LU1	866.67	81.42	128.79	4.29	5.42	10.65	9.71	9.86	6.67	
ACT	LU2	47.54		82.40	1.96	1.43	8.57	9.19	4.88	3.47	
ACT	LU3	212.04	68.75	79.08	1.36	3.98	8.52	4.16	4.17	0.82	

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI or A+T = Activation: Homogenate Control (Liver) ALU = Activation: Homogenate Control (Lung) ACP = Activation: Positive Control ACT = Activation Test LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = x 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (Macaca mulatta)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, Powdered Guaiac Resin, FDA 75-66, was evaluation for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate tests

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative. The LI1 and LU1 doses with mouse and rat tissues and LI1 dose with monkey tissue were repeated as these doses appeared to be toxic in the initial test. The LU2 dose with rat tissue and LU1 and LU3 doses with monkey tissue showed higher revertant frequency in the initial test. The repeat test with these doses was negative.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

The test compound, Powdered Guaiac Resin, FDA 75-66, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

David J. Brusick, Ph.D.
Director
Department of Genetics

4/18/77
Date

Reviewed by:

Robert J. Weir, Ph.D.
Vice President

4/18/77
Date

VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the *his*$^-$ cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

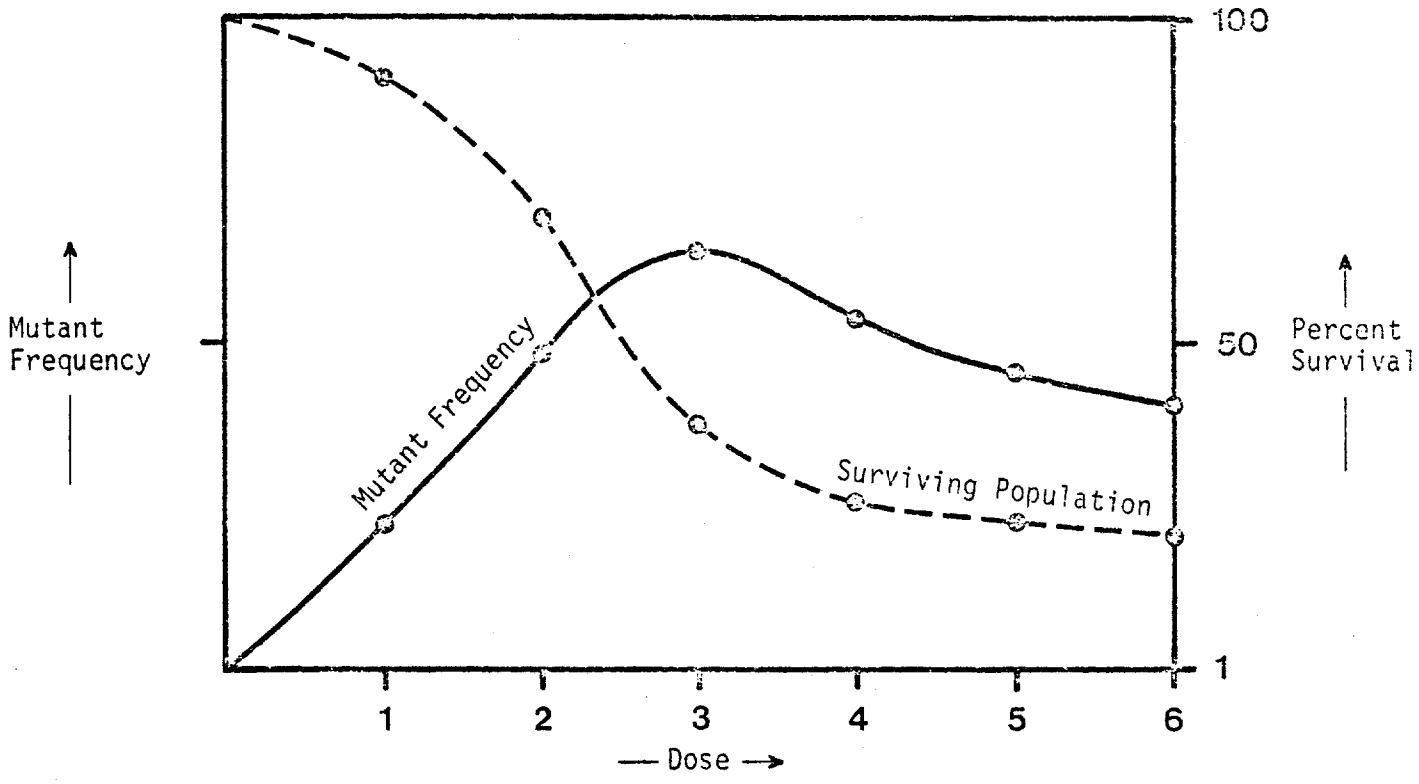
D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT	OBSERVED DOSE RESPONSE
(1) Dose levels 1,2 & 3 were used	A typical positive dose response set of data would be obtained.
(2) Dose levels 2, 3 & 4 were used	The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
(3) Dose levels 3, 4 & 5 were used	Here an inverted dose response would be observed with the highest dose level showing the lowest response.

APPENDIX

Tabulation of Data

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 633704 DETECTOR TA100 SPECIES / DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0797	0447	56.09	0
	NAP		EMS 0.066%	0885	1455	164.41	0
PM9000297	NA1		0022-2 PCT.	0323	0142	43.96	0
PM9000297	NA2		0011-2 PCT.	0537	0260	48.42	0
PM9000297	NA3		0055-3 PCT.	0676	0321	47.49	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 633402 DETECTOR TA1535 SPECIES / DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	N4N		SOLVENT	0543	0029	5.34	0
	NAP		EMS 0.2%	0633	3718	587.36	0
PM9000297	NA1		0022-2 PCT.	0392	0020	5.10	0
PM9000297	NA2		0011-2 PCT.	0457	0027	5.91	0
PM9000297	NA3		0055-3 PCT.	0824	0028	3.40	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 633404 DETECTOR TA1537 SPECIES / DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0643	0129	20.06	0
	NAP		QM 13 UG/ML	0240	0331	137.92	0
PM9000297	NA1		0022-2 PCT.	1321	0268	20.29	0
PM9000297	NA2		0011-2 PCT.	1727	0247	14.30	0
PM9000297	NA3		0055-3 PCT.	1477	0220	14.90	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 633403 DETECTOR TA1538 SPECIES / DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0456	0050	10.96	0
	NAP		NF 667 UG/ML	0458	0857	187.12	0
PM9000297	NA1		0022-2 PCT.	0423	0042	9.93	0
PM9000297	NA2		0011-2 PCT.	0487	0048	9.86	0
PM9000297	NA3		0055-3 PCT.	0450	0045	10.00	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634101 DETECTOR TA98 SPECIES / DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	N4N		SOLVENT	0501	0027	5.39	0
	NAP		NF 667 UG/ML	1255	1801	143.51	0
PM9000297	NA1		0022-2 PCT.	0731	0052	7.11	0
PM9000297	NA2		0011-2 PCT.	0664	0028	4.22	0
PM9000297	NA3		0055-3 PCT.	1200	0040	3.33	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 633405 DETECTOR 0000D4 SPECIES / DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	1841	0126	0158	6.84	8.58	0
	NAP		EMS 1.0 %	0733	1027	1559	249.25	212.69	0
PM9000297	NA1		0125-2 PCT.	1032	0130	0076	12.60	7.36	0
PM9000297	NA2		0625-3 PCT.	1475	0120	0162	8.14	10.98	0
PM9000297	NA3		3125-4 PCT.	1471	0126	0084	8.57	5.71	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 633602 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	1301	0562	43.20	0
	A-C		SOLVENT	1508	0491	32.56	0
	ALI		TISSUE	1613	0515	31.93	0
	ALU		TISSUE	1564	0694	44.37	0
	ACP	LI	DMN 90 UM/ML	1411	0522	37.00	0
	ACP	LU	DMN 90 UM/ML	1768	0483	27.32	0
PM9000297	ACT	LI1	0022-2 PCT.	0000	0019	******	0
PM9000297	ACT	LI2	0011-2 PCT.	0213	0141	66.20	0
PM9000297	ACT	LI3	0055-3 PCT.	1064	0422	39.66	0
PM9000297	ACT	LU1	0022-2 PCT.	0001	0033	******	0
PM9000297	ACT	LU2	0011-2 PCT.	0786	0152	19.34	0
PM9000297	ACT	LU3	0055-3 PCT.	1429	0527	36.88	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708001 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-A	CONTAM
	A+C		DMN 90 UM/ML	1082	0641	59.24	0
	A-C		SOLVENT	1151	0543	47.18	0
	ALI		TISSUE	1464	0659	45.01	0
	ALU		TISSUE	0912	0682	74.78	0
	ACP	LI	DMN 90 UM/ML	1333	1401	105.10	0
	ACP	LU	DMN 90 UM/ML	1311	0752	57.36	0
PM9000297	ACT	LI1	0022-2 PCT.	0448	0281	62.72	0
PM9000297	ACT	LI2	0011-2 PCT.	0904	0651	72.01	0
PM9000297	ACT	LI3	0055-3 PCT.	1533	0731	47.68	0
PM9000297	ACT	LU1	0022-2 PCT.	0461	0252	54.66	0
PM9000297	ACT	LU2	0011-2 PCT.	1099	0642	58.42	0
PM9000297	ACT	LU3	0055-3 PCT.	1019	0648	63.59	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 633501 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0433	0039	9.01	0
	A-C		SOLVENT	0444	0037	8.33	0
	ALI		TISSUE	0459	0051	11.11	0
	ALU		TISSUE	0447	0039	8.72	0
	ACP	LI	DMN 90 UM/ML	0445	0800	179.78	0
	ACP	LU	DMN 90 UM/ML	0416	0049	11.78	0
PM9000297	ACT	LI1	0022-2 PCT.	0436	0035	8.03	0
PM9000297	ACT	LI2	0011-2 PCT.	0908	0051	5.62	0
PM9000297	ACT	LI3	0055-3 PCT.	1020	0050	4.90	0
PM9000297	ACT	LU1	0022-2 PCT.	0544	0018	3.31	0
PM9000297	ACT	LU2	0011-2 PCT.	0911	0041	4.50	0
PM9000297	ACT	LU3	0055-3 PCT.	1134	0049	4.32	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634105 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMO 333 UG/ML	1535	0065	4.23	0
	A-C		SOLVENT	1346	0032	2.38	0
	ALI		TISSUE	0884	0029	3.28	0
	ALU		TISSUE	0372	0021	5.65	0
	ACP	LI	AMO 333 UG/ML	0615	0413	67.15	0
	ACP	LU	AMO 333 UG/ML	1808	0033	1.83	0
PM9000297	ACT	LI1	0022-2 PCT.	0391	0024	6.14	0
PM9000297	ACT	LI2	0011-2 PCT.	1380	0025	1.81	0
PM9000297	ACT	LI3	0055-3 PCT.	1130	0022	1.95	0
PM9000297	ACT	LU1	0022-2 PCT.	0436	0018	4.13	0
PM9000297	ACT	LU2	0011-2 PCT.	1483	0027	1.82	0
PM9000297	ACT	LU3	0055-3 PCT.	1760	0035	1.99	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 633502 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0304	0037	12.17	0
	A-C		SOLVENT	0352	0026	7.39	0
	ALI		TISSUE	0434	0047	10.83	0
	ALU		TISSUE	0446	0052	11.66	0
	ACP	LI	ANTH 67 UG/ML	0433	0579	133.72	0
	ACP	LU	ANTH 67 UG/ML	0419	0179	42.72	0
PM9000297	ACT	LI1	0022-2 PCT.	0161	0010	6.21	0
PM9000297	ACT	LI2	0011-2 PCT.	0152	0010	6.58	0
PM9000297	ACT	LI3	0055-3 PCT.	0437	0045	10.30	0
PM9000297	ACT	LU1	0022-2 PCT.	0114	0012	10.53	0
PM9000297	ACT	LU2	0011-2 PCT.	0141	0011	7.80	0
PM9000297	ACT	LU3	0055-3 PCT.	0415	0056	13.49	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634104 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1605	0041	2.55	0
	A-C		SOLVENT	1531	0044	2.87	0
	ALI		TISSUE	1051	0061	5.80	0
	ALU		TISSUE	0941	0047	4.99	0
	ACP	LI	ANTH 67 UG/ML	1181	0685	58.00	0
	ACP	LU	ANTH 67 UG/ML	1010	0252	24.95	0
PM9000297	ACT	LI1	0022-2 PCT.	0337	0063	18.69	0
PM9000297	ACT	LI2	0011-2 PCT.	1115	0056	5.02	0
PM9000297	ACT	LI3	0055-3 PCT.	1113	0051	4.58	0
PM9000297	ACT	LU1	0022-2 PCT.	0389	0039	10.03	0
PM9000297	ACT	LU2	0011-2 PCT.	0797	0054	6.78	0
PM9000297	ACT	LU3	0055-3 PCT.	0934	0036	3.85	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634901 DETECTOR 000004 SPECIES ICRFLO/MOUSE DATE - 04/12/77

COMPOUND	TEST	OPG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1194	0114	0052	9.55	4.36	0
	A-C		SOLVENT	0600	0057	0010	9.50	1.67	0
	ALI		TISSUE	0407	0084	0036	20.64	8.85	0
	ALU		TISSUE	0912	0058	0014	6.36	1.54	0
	ACP	LI	DMN 90 UM/ML	1366	0831	0742	60.83	54.32	0
	ACP	LU	DMN 90 UM/ML	1044	0092	0042	8.81	4.02	0
PM9000297	ACT	LI1	0125-2 PCT.	0812	0073	0050	8.99	6.16	0
PM9000297	ACT	LI2	0625-3 PCT.	1021	0147	0083	14.40	8.13	0
PM9000297	ACT	LI3	3125-4 PCT.	1085	0060	0033	5.53	3.04	0
PM9000297	ACT	LU1	0125-2 PCT.	1026	0141	0050	13.74	4.87	0
PM9000297	ACT	LU2	0625-3 PCT.	1276	0114	0063	8.93	4.94	0
PM9000297	ACT	LU3	3125-4 PCT.	1472	0139	0086	9.44	5.84	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 633603 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	1249	0574	45.96	0
	A-C		SOLVENT	1016	0500	49.21	0
	ALI		TISSUE	1275	0635	49.80	0
	ALU		TISSUE	1203	0632	52.54	0
	ACP	LI	DMN 90 UM/ML	1111	0516	46.44	0
	ACP	LU	DMN 90 UM/ML	1349	0525	38.92	0
PM9000297	ACT	LI1	0022-2 PCT.	0000	0009	******	0
PM9000297	ACT	LI2	0011-2 PCT.	1053	0547	51.95	0
PM9000297	ACT	LI3	0055-3 PCT.	1069	0508	47.52	0
PM9000297	ACT	LU1	0022-2 PCT.	0001	0023	******	0
PM9000297	ACT	LU2	0011-2 PCT.	0042	0059	140.48	0
PM9000297	ACT	LU3	0055-3 PCT.	1259	0599	47.58	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634303 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	ALI		TISSUE	0787	0563	71.54	0
	ALU		TISSUE	0890	0576	64.72	0
	ACP	LI	OMN 90 UM/ML	0460	0348	75.65	0
PM9000297	ACT	LI1	0022-2 PCT.	0546	0289	52.93	0
PM9000297	ACT	LU1	0022-2 PCT.	0447	0289	64.65	0
PM9000297	ACT	LU2	0011-2 PCT.	0493	0250	50.71	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 703501 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0541	0729	134.75	0
	A-C		SOLVENT	0674	0704	104.45	0
	ALI		TISSUE	0636	0880	138.36	0
	ALU		TISSUE	0533	0701	131.52	0
	ACP	LI	DMN 90 UM/ML	0590	0946	160.34	0
	ACP	LU	DMN 90 UM/ML	0785	0759	96.69	0
PM9000297	ACT	LI1	0022-2 PCT.	0751	0534	71.11	0
PM9000297	ACT	LI2	0011-2 PCT.	0989	0813	82.20	0
PM9000297	ACT	LI3	0055-3 PCT.	0990	1036	104.65	0
PM9000297	ACT	LU1	0022-2 PCT.	0493	0624	126.57	0
PM9000297	ACT	LU2	0011-2 PCT.	0849	0724	85.28	0
PM9000297	ACT	LU3	0055-3 PCT.	0741	0773	104.32	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 633601 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 04/12/77

COMPOUND	TEST	OPG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0928	0063	6.79	0
	A-C		SOLVENT	0758	0066	8.71	0
	ALI		TISSUE	1013	0070	6.91	0
	ALU		TISSUE	1024	0071	6.93	0
	ACP	LI	DMN 90 UM/ML	0855	0731	85.50	0
	ACP	LU	DMN 90 UM/ML	1160	0074	6.38	0
PM9000297	ACT	LI1	0022-2 PCT.	0394	0018	4.57	0
PM9000297	ACT	LI2	0011-2 PCT.	1506	0081	5.38	0
PM9000297	ACT	LI3	0055-3 PCT.	1539	0046	2.99	0
PM9000297	ACT	LU1	0022-2 PCT.	0299	0011	3.68	0
PM9000297	ACT	LU2	0011-2 PCT.	1037	0076	7.33	0
PM9000297	ACT	LU3	0055-3 PCT.	0906	0052	5.74	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634203 DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP*6	MUTI EP*0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0675	0073	10.81	0
	A-C		SOLVENT	0986	0031	3.14	0
	ALI		TISSUE	0750	0027	3.60	0
	ALU		TISSUE	0372	0026	6.99	0
	ACP	LI	AMQ 333 UG/ML	0523	0234	44.74	0
	ACP	LU	AMQ 333 UG/ML	1187	0067	5.64	0
PM9000297	ACT	LI1	0022-2 PCT.	0352	0029	8.24	0
PM9000297	ACT	LI2	0011-2 PCT.	1433	0021	1.47	0
PM9000297	ACT	LI3	0055-3 PCT.	1379	0017	1.23	0
PM9000297	ACT	LU1	0022-2 PCT.	0565	0015	2.65	0
PM9000297	ACT	LU2	0011-2 PCT.	1283	0033	2.57	0
PM9000297	ACT	LU3	0055-3 PCT.	1321	0028	2.12	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 633801 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0133	0031	23.31	0
	A-C		SOLVENT	0188	0020	10.64	0
	ALI		TISSUE	0267	0017	6.37	0
	ALU		TISSUE	0164	0011	6.71	0
	ACP	LI	ANTH 67 UG/ML	0232	0054	23.28	0
	ACP	LU	ANTH 67 UG/ML	0153	0866	566.01	0
PM9000297	ACT	LI1	0022-2 PCT.	0490	0043	8.78	0
PM9000297	ACT	LI2	0011-2 PCT.	0397	0016	4.03	0
PM9000297	ACT	LI3	0055-3 PCT.	0335	0029	8.66	0
PM9000297	ACT	LU1	0022-2 PCT.	0429	0046	10.72	0
PM9000297	ACT	LU2	0011-2 PCT.	0509	0017	3.34	0
PM9000297	ACT	LU3	0055-3 PCT.	0590	0022	3.73	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 633801 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0133	0031	23.31	0
	A-C		SOLVENT	0188	0020	10.64	0
	ALI		TISSUE	0267	0017	6.37	0
	ALU		TISSUE	0164	0011	6.71	0
	ACP	LI	ANTH 67 UG/ML	0232	0054	23.28	0
	ACP	LU	ANTH 67 UG/ML	0153	0866	566.01	0
PM9000297	ACT	LI1	0022-2 PCT.	0490	0043	8.78	0
PM9000297	ACT	LI2	0011-2 PCT.	0397	0016	4.03	0
PM9000297	ACT	LI3	0055-3 PCT.	0335	0029	8.66	0
PM9000297	ACT	LU1	0022-2 PCT.	0429	0046	10.72	0
PM9000297	ACT	LU2	0011-2 PCT.	0509	0017	3.34	0
PM9000297	ACT	LU3	0055-3 PCT.	0590	0022	3.73	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634204 DETECTOR TA98 SPECIES SPRDAW/RAT DATE - 04/12/77

COMPOUND	TEST	OPG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1972	0074	3.75	0
	A-C		SOLVENT	1309	0048	3.67	0
	ALI		TISSUE	0582	0061	10.48	0
	ALU		TISSUE	0886	0057	6.43	0
	ACP	LI	ANTH 67 UG/ML	1599	1757	109.88	0
	ACP	LU	ANTH 67 UG/ML	0678	0585	86.28	0
PM9000297	ACT	LI1	0022-2 PCT.	0314	0055	17.52	0
PM9000297	ACT	LI2	0011-2 PCT.	0865	0058	6.71	0
PM9000297	ACT	LI3	0055-3 PCT.	0858	0060	6.99	0
PM9000297	ACT	LU1	0022-2 PCT.	0950	0062	6.53	0
PM9000297	ACT	LU2	0011-2 PCT.	1599	0059	3.69	0
PM9000297	ACT	LU3	0055-3 PCT.	1115	0053	4.75	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634903 DETECTOR 0000D4 SPECIES SPRDAW/RAT DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1526	0105	0082	6.88	5.37	0
	A-C		SOLVENT	1548	0098	0064	6.33	4.13	0
	ALI		TISSUE	1009	0128	0056	12.69	5.55	0
	ALU		TISSUE	1357	0119	0067	8.77	4.94	0
	ACP	LI	DMN 90 UM/ML	1199	0895	0771	74.65	64.30	0
	ACP	LU	DMN 90 UM/ML	1407	0125	0054	8.88	3.84	0
PM9000297	ACT	LI1	0125-2 PCT.	1085	0122	0046	11.24	4.24	0
PM9000297	ACT	LI2	0625-3 PCT.	1762	0090	0070	5.11	3.97	0
PM9000297	ACT	LI3	3125-4 PCT.	1750	0113	0065	6.46	3.71	0
PM9000297	ACT	LU1	0125-2 PCT.	1000	0111	0042	11.10	4.20	0
PM9000297	ACT	LU2	0625-3 PCT.	1544	0112	0077	7.25	4.99	0
PM9000297	ACT	LU3	3125-4 PCT.	1296	0105	0084	8.10	6.48	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 633701 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	1210	0621	51.32	0
	A-C		SOLVENT	1727	0566	32.77	0
	ALI		TISSUE	1522	0688	45.20	0
	ALU		TISSUE	1751	0797	45.52	0
	ACP	LI	DMN 90 UM/ML	1530	0604	39.48	0
	ACP	LU	DMN 90 UM/ML	1466	0607	41.41	0
PM9000297	ACT	LI1	0022-2 PCT.	0000	0086	******	0
PM9000297	ACT	LI2	0011-2 PCT.	0190	0139	73.16	0
PM9000297	ACT	LI3	0055-3 PCT.	1532	0587	38.32	0
PM9000297	ACT	LU1	0022-2 PCT.	0003	0026	866.67	0
PM9000297	ACT	LU2	0011-2 PCT.	0345	0164	47.54	0
PM9000297	ACT	LU3	0055-3 PCT.	0216	0458	212.04	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634807 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	ALI		TISSUE	1298	0411	31.66	0
	ALU		TISSUE	1428	0410	28.71	0
	ACP	LI	OMN 90 UM/ML	1064	0878	82.52	0
PM9000297	ACT	LI1	0022-2 PCT.	0327	0222	67.89	0
PM9000297	ACT	LU1	0022-2 PCT.	0253	0206	81.42	0
PM9000297	ACT	LU3	0055-3 PCT.	0368	0253	68.75	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 703502 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0639	0786	123.00	0
	A-C		SOLVENT	0536	0460	85.82	0
	ALI		TISSUE	0965	0880	91.19	0
	ALU		TISSUE	0826	0867	104.96	0
	ACP	LI	DMN 90 UM/ML	1031	2250	218.23	0
	ACP	LU	DMN 90 UM/ML	1228	0980	79.80	0
PM9000297	ACT	LI1	0022-2 PCT.	0652	0643	98.62	0
PM9000297	ACT	LI2	0011-2 PCT.	1039	0925	89.03	0
PM9000297	ACT	LI3	0055-3 PCT.	0950	0776	81.68	0
PM9000297	ACT	LU1	0022-2 PCT.	0455	0586	128.79	0
PM9000297	ACT	LU2	0011-2 PCT.	0818	0674	82.40	0
PM9000297	ACT	LU3	0055-3 PCT.	0865	0684	79.08	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634A01 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0510	0046	9.02	0
	A-C		SOLVENT	1088	0029	2.67	0
	ALI		TISSUE	1289	0028	2.17	0
	ALU		TISSUE	0953	0033	3.46	0
	ACP	LI	DMN 90 UM/ML	0754	0888	117.77	0
	ACP	LU	DMN 90 UM/ML	0308	0139	45.13	0
PM9000297	ACT	LI1	0022-2 PCT.	0561	0018	3.21	0
PM9000297	ACT	LI2	0011-2 PCT.	1099	0028	2.55	0
PM9000297	ACT	LI3	0055-3 PCT.	1180	0021	1.78	0
PM9000297	ACT	LU1	0022-2 PCT.	0420	0018	4.29	0
PM9000297	ACT	LU2	0011-2 PCT.	1072	0021	1.96	0
PM9000297	ACT	LU3	0055-3 PCT.	1099	0015	1.36	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634301 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1787	0039	2.18	0
	A-C		SOLVENT	1294	0112	8.66	0
	ALI		TISSUE	0792	0030	3.79	0
	ALU		TISSUE	0814	0034	4.18	0
	ACP	LI	AMQ 333 UG/ML	0486	0350	72.02	0
	ACP	LU	AMQ 333 UG/ML	0858	0031	3.61	0
PM9000297	ACT	LI1	0022-2 PCT.	0439	0022	5.01	0
PM9000297	ACT	LI2	0011-2 PCT.	1261	0032	2.54	0
PM9000297	ACT	LI3	0055-3 PCT.	1604	0036	2.24	0
PM9000297	ACT	LU1	0022-2 PCT.	0498	0027	5.42	0
PM9000297	ACT	LU2	0011-2 PCT.	1962	0028	1.43	0
PM9000297	ACT	LU3	0055-3 PCT.	1105	0044	3.98	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634201 DETECTOR TA1538 SPECIES RHESUS/MONKEY DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0697	0065	9.33	0
	A-C		SOLVENT	0700	0053	7.57	0
	ALI		TISSUE	0670	0050	7.46	0
	ALU		TISSUE	0648	0054	8.33	0
	ACP	LI	ANTH 67 UG/ML	0277	0621	224.19	0
	ACP	LU	ANTH 67 UG/ML	0577	0060	10.40	0
PM9000297	ACT	LI1	0022-2 PCT.	0481	0052	10.81	0
PM9000297	ACT	LI2	0011-2 PCT.	0504	0051	10.12	0
PM9000297	ACT	LI3	0055-3 PCT.	0515	0054	10.49	0
PM9000297	ACT	LU1	0022-2 PCT.	0479	0051	10.65	0
PM9000297	ACT	LU2	0011-2 PCT.	0572	0049	8.57	0
PM9000297	ACT	LU3	0055-3 PCT.	0575	0049	8.52	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634202 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	2094	0077	3.68	0
	A-C		SOLVENT	1656	0093	5.62	0
	ALI		TISSUE	0563	0077	13.68	0
	ALU		TISSUE	0949	0069	7.27	0
	ACP	II	ANTH 67 UG/ML	1006	1009	100.30	0
	ACP	IU	ANTH 67 UG/ML	0875	0076	8.69	0
PM9000297	ACT	LII	0022-2 PCT.	0506	0064	12.65	0
PM9000297	ACT	LI2	0011-2 PCT.	1638	0068	4.15	0
PM9000297	ACT	LI3	0055-3 PCT.	1181	0074	6.27	0
PM9000297	ACT	LUI	0022-2 PCT.	0618	0060	9.71	0
PM9000297	ACT	LU2	0011-2 PCT.	0892	0082	9.19	0
PM9000297	ACT	LU3	0055-3 PCT.	1635	0068	4.16	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 634904 DETECTOR 000004 SPECIES RHESUS/MONKEY DATE - 04/12/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1299	0081	0053	6.24	4.08	0
	A-C		SOLVENT	1425	0070	0067	4.91	4.70	0
	ALI		TISSUE	1211	0092	0097	7.60	8.01	0
	ALU		TISSUE	1505	0099	0079	6.58	5.25	0
	ACP	LI	DMN 90 UM/ML	1733	0893	0874	51.53	50.43	0
	ACP	LU	DMN 90 UM/ML	1573	0098	0066	6.23	4.20	0
PM9000297	ACT	LI1	0125-2 PCT.	0684	0068	0046	9.94	6.73	0
PM9000297	ACT	LI2	0625-3 PCT.	1094	0061	0064	5.58	5.85	0
PM9000297	ACT	LI3	3125-4 PCT.	1799	0068	0004	3.78	0.22	0
PM9000297	ACT	LU1	0125-2 PCT.	0720	0071	0048	9.86	6.67	0
PM9000297	ACT	LU2	0625-3 PCT.	1701	0083	0059	4.88	3.47	0
PM9000297	ACT	LU3	3125-4 PCT.	1584	0066	0013	4.17	0.82	0